swimming in the same type of still waters (Lake Malawi for *S. haematobium* and Burkina Faso for *S. mansoni*). It must be kept in mind that location of the eggs in the spinal cord is due to aberrant migration, so it is rather surprising to note this rare phenomenon in our two patients. It has been previously reported that differences in schistosomiasis-associated morbidity may be observed between areas where prevalence and intensities of infection are comparable [10]. The reasons for these differences are still not clear.

The differences between patients depend on many factors, such as intensity and duration of infection, nutritional state, concomitant infections, differences in pathogenicity between local parasite strains, and host genetic differences [11]. Since the two patients were Caucasian, non-immune and in a normal nutritional state, and since they contracted schistosomiasis under similar conditions, it may be suggested that the spinal cord localization is either a random accident or due to a particular neurotropism of the strain. Intraspecific variation is widespread amongst *Schistosoma* spp. resulting in differences in infection rate, egg distribution in tissues, maturation time, egg-laying capacity, pathogenicity, virulence, and susceptibility to drugs [12,13]. These differences may directly affect the epidemiology of schistosomiasis in various geographic areas. It would be of great interest to analyze the genomic patterns of strains isolated from areas where clinical manifestations are atypical.

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References

1. Scrimgeour EM, Gajdusek DC. Involvement of the central nervous system in *Schistosoma mansoni* and *S. haematobium* infection. Brain 1985; 108: 1023–38.
2. Lechtenberg R, Vaida GA. Schistosomiasis of the spinal cord. Neurology 1977; 27: 55–9.
3. Queiroz L, Nucci A, Facure N, Facure J. Massive spinal cord necrosis in schistosomiasis. Arch Neurol 1979; 36: 517–19.
4. Case records of the Massachusetts General Hospital (Case 4-1996). N Engl J Med 1996; 334: 382–9.
5. Dupuis JM, Atroni S, Dooms GC, Gonsette RE. MRI imaging of schistosomal myelitis. AJNR 1990; 11: 782–3.
6. El-Banhawy A, Elwan O, Taher Y. Bilharzial granuloma of the conus medullaris and cauda equina. Paraplegia 1972; 10: 172–80.
7. Wofe M, Parenti D, Pollner J, Kabrine A, Schwartz A. Schistosomiasis in US Peace Corps volunteers—Malawi, 1992. MMWR 1993; 42: 565–70.
8. Blunt SB, Boulton J, Wise R. MRI in schistosomiasis of conus medullaris and lumbar spinal cord. Lancet 1993; 341: 557.
9. Pollner J, Schwartz A, Kabrine A, Parenti D. Cerebral schistosomiasis caused by *Schistosoma haematobium*: case report. Clin Infect Dis 1994; 18: 354–7.
10. Thompson RCA. Intraspecific variation and epidemiology. In Mehlhorn H, ed. Parasitology in focus. Berlin: Springer-Verlag, 1988: 391–411.
11. Theron A. Early and late shedding patterns of *Schistosoma mansoni* cercariae: ecological significance in transmission to human and murine hosts. J Parasitol 1984; 70: 652–5.
12. Cheever AW. *Schistosoma japonicum*: the pathology of experimental infection. Exp Parasitol 1985; 59: 1–11.
13. Soliman GN, El Assal FM, Monsour NS, Garo K. Comparison of two Egyptian strains of *Schistosoma mansoni* in hamsters. Z Parasitenkd 1986; 72: 353–63.

Acrodermatitis chronica atrophicans and serologic confirmation of infection due to *Borrelia afzelii* and/or *Borrelia garinii* by immunoblot

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Acrodermatitis chronica atrophicans (ACA) is a late chronic cutaneous manifestation of Lyme borreliosis [1], recognized almost exclusively in Europe [2]. Since 1992, European isolates of *Borrelia burgdorferi* sensu lato, the agent causing Lyme disease have been subdivided into three major genospecies: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* [2,3]. Whereas *B. burgdorferi* sensu stricto seems to be the major if not the only genospecies of clinical importance in North America, European isolates belong to three genospecies, mostly *B. garinii* or *B. afzelii* [4]. Recent findings suggest that these three genospecies are associated with different late clinical manifestations, respectively to Lyme arthritis, neuroborreliosis and ACA [2,5–7].

Because of its sometimes debilitating characteristics and its poor prognosis, ACA should be diagnosed and treated as soon as possible. Although *B. burgdorferi* sensu lato has been successfully cultured from various clinical lesions, culture remains a difficult procedure that is not practicable for routine diagnosis. Clinical diagnosis

is currently supported by measuring the antibody response to the spirochete: indirect immunofluorescence (IF) or enzyme-linked immunosorbent assays (ELISA) are routinely used. In these screening tests, whole cells or whole cell sonicated extract are used as antigens; this may give rise to false-positive results due to cross-reaction with irrelevant antigens in the preparation. Apart from the problem of specificity, which can be partly resolved with ELISAs which use purified antigens such as the flagellum or the OspC or recombinant antigens such as p39 or an internal fragment of flagellin, these tests entail a loss of sensitivity in the specific antibody response to a single *Borrelia* species.

The aim of the study was to develop a type-specific immunoblot assay using whole cell lysates of each of the three genospecies to analyze the serologic response of patients with ACA. A total of 25 patients were retrospectively enrolled on the basis of dermatologic symptoms compatible with ACA and a positive screening test by ELISA (VIDAS Lyme IgG + IgM BioMérieux, France). Results were confirmed by immunoblot using the detection of specific IgG to the three species of *Borrelia*. All 25 patient sera were screened for antibody to *B. burgdorferi* sensu lato using five other commercial ELISA tests.

The isolates used as antigens in the immunoblot assays were: VS 215 (*B. burgdorferi* sensu stricto), VS 102 (*B. garinii*), and VS 461 (*B. afzelii*). Isolates used in this study were all low-passage strains (fewer than nine passages in BSK-II medium) isolated from *Ixodes ricinus* [8]. The three isolates were typed by phenotypic analysis [8] and genotypic analysis according to the method of Postic et al [9].

The SDS-PAGE and immunoblot assays were performed as previously described [8]. The criterion for a positive immunoblot with human serum was at least five bands including flagellin and two of the following specific bands: OspC, OspA, p39, 93 kDa (p100), as previously described [7]. Immunoblots for the three species were performed with three strips, one for each species (VS 215, *B. burgdorferi* sensu stricto, VS 102, *B. garinii*, and VS 461, *B. afzelii*). They were prepared and incubated with serum from each patient and were processed through all stages in the same strip tray.

For comparison between the three immunoblots, scores were allocated (0–3 points) according to the presence and intensity of the reaction to seven borrelia proteins, as shown in Figure 1(a,b), 93 kDa (p100), flagellin, p39, OspA, OspD, OspC and 18 kDa. This scoring system has been described and validated for neuroborreliosis [7]. A total score superior by two points for one individual species compared with the other species was defined as a specific reaction, based on a statistical analysis previously described [7] (for example, B 10, A 18, G 11 was typed as *B. afzelii*). This criterion was based on the significant differences observed in the statistical analysis of the mean scores.

The sera of 23 patients with ACA were positive with six commercial ELISAs, while two sera were unreactive, each with one different ELISA, even though they were strongly reactive with the other five ELISAs (Table 1).

The overall total scores established for the 25 patients by immunoblot were as follows: 403 points for *B. afzelii*, 319 points for *B. garinii* and 271 points for *B. burgdorferi* sensu stricto. Among the 25 patients reacting specifically with *B. burgdorferi* sensu lato, 20, clearly demonstrated a stronger reaction to *B. afzelii* (80%) (Figure 1a), with a mean score of 4 above the other two species. Obvious specific reactions to *B. garinii* were observed in two patients (8%) (Table 1, nos. 4 and 9; Figure 1b) and reactions to both *B. afzelii* and *B. garinii* were found in three patients (12%) (Table 1, nos. 3, 11 and 16). No preferential reactivity to *B. burgdorferi* sensu stricto was observed.

This study clearly demonstrated the association between *B. afzelii* and ACA. Differentiation of the specific reactivity was quite obvious in the immunoblots, as observed by Assous et al [6]. The numerical scores allowed us to define the specific reactivities on a semiquantitative basis. In 20 of 25 patients (80%) the score was definitely higher for *B. afzelii*. In three additional patients we may suspect on the basis of the serologic reactions a dual infection by *B. afzelii* and *B. garinii*, as previously described by Demaerschalk et al [10] in patients with neuroborreliosis. Two of the 25 cases (8%) showed an unequivocally stronger reaction to *B. garinii*. Recently, Rijpkema et al [11] amplified by polymerase chain reaction (PCR) *B. garinii* genomic material from the skin of patients with ACA and also detected mixed infection by *B. afzelii* and *B. garinii*. These results seem to indicate that the association between particular *Borrelia* genospecies and clinical manifestations is real but not absolute. Unfortunately, no skin biopsy was available for PCR confirmation in the two above mentioned cases.

A similar association between *B. garinii* and neuroborreliosis was observed among a group of 28 patients with neuroborreliosis. In this study [7], we used the same immunoblots, and we showed that scores were higher for *B. garinii* (353 points) than for *B. afzelii* (291 points) and for *B. burgdorferi* sensu stricto (283 points). Overall, 64% (18/28) of these patients with neuroborreliosis showed a stronger antibody reaction to *B. garinii*, 11% (3/28) to *B. burgdorferi* sensu stricto and 7% (2/28) to *B. afzelii*. These results were significantly

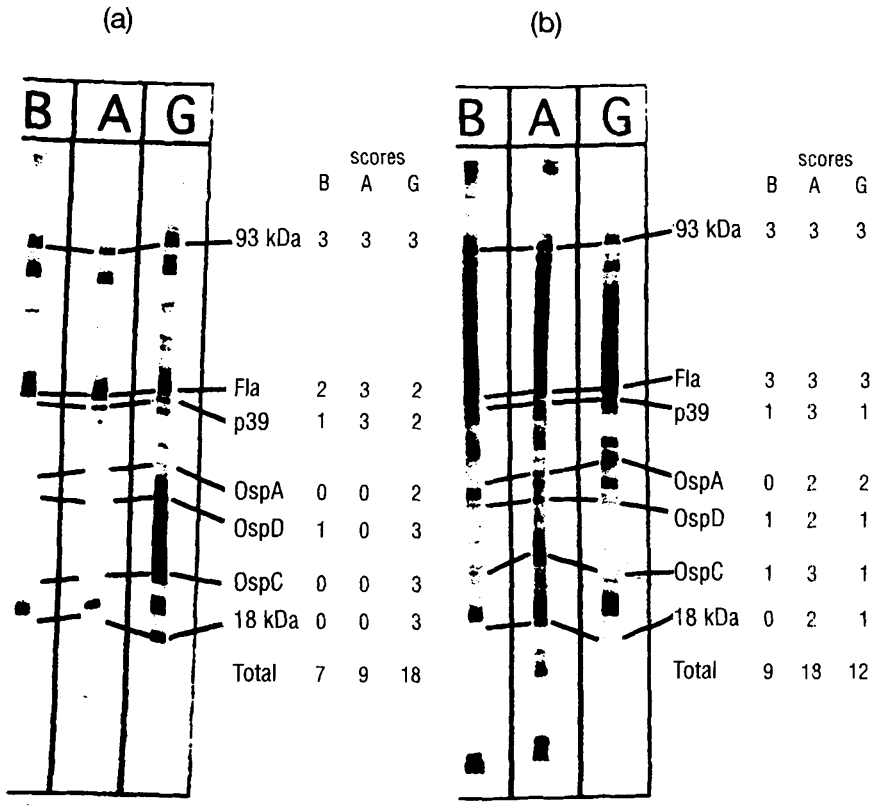

Figure 1a) Example of predominant score to *Borrelia afzelii* (A). **Figure 1b)** Example of predominant score to *Borrelia garinii* (G). Immunoblots (IgG) with the serum of patients with ACA and three strips: B: VS 215, *B. burgdorferi* sensu stricto; A: VS 461, *B. afzelii*, G: VS 102, *B. garinii*. Score points are allocated to 93-kDa protein, flagellin, p39, OspA, OspD, OspC and 18-kDA protein.

different ($p<0.02$) from those of a control group of 20 patients with erythema migrans.

Although the diagnosis of Lyme disease is primarily based on clinical manifestations, laboratory data are frequently necessary to elucidate borderline clinical presentations. The direct detection of the causative agent is impaired by the low number of borreliae present in tissue and especially in body fluids. Even the highly sensitive PCR seems not to be superior to the conventional culture isolation methods [12], except for synovial fluid, from which borreliae have rarely been isolated but have been demonstrated by PCR in up to 96% of untreated Lyme arthritis cases [13]. Serologic techniques are, therefore, the most widely used methods to establish the diagnosis of Lyme borreliosis. Several attempts to increase the sensitivity and specificity of serologic tests have included absorption procedures with *Treponema phagedenis*, use of purified antigens or recombinant antigens and the association of different recombinant antigens selected from different strains in the same immunoblot [14]. The four antigens studied by Wilske et al were flagellin, an internal flagellin fragment, the 93-kDa protein called p100, the plasmid-encoded outer-surface proteins OspA and OspC. The authors concluded that recombinant antigens from different genospecies of *B. burgdorferi* sensu lato are serologically different, and that specific clinical manifestations are correlated with each of the three genospecies. Therefore, genospecific tests for each clinical indication could provide more sensitivity and specificity than a single common test.

The type-specific assay described here is based on seven antigens—93 kDa or p100, flagellin, p39, OspA, OspD, OspC and 18-kDa proteins specifically recognized in each of the three genospecies. In this study, each serum was tested for the presence of specific IgG against all these antigenic proteins of each of the three genospecies. The serologic data in the present study show a clear association of *B. afzelii* with ACA and confirm the results of isolations typed as *B. afzelii* from the skin of patients with ACA [2,15]. Furthermore, our data strongly suggest that *B. garinii* is also able to produce chronic dermatologic disorders such as ACA, though at a much lower frequency than *B. afzelii*. This is, to the best of our knowledge, the first report of serologic determination of *B. garinii* as the

Table 1 Serologic results of 25 patients with acrodermatitis chronica atrophicans

Patient no.	ELISA	Immunoblot scores B	A	G	Interpretation
1	+++	12	14	11	*B. afzelii*
2	+++	13	18	12	*B. afzelii*
3	+++	6	15	14	*B. afzelii–B. garinii*
4	+++	13	14	18	*B. garinii*
5	+++	12	18	14	*B. afzelii*
6	++	12	19	12	*B. afzelii*
7	+++	8	13	10	*B. afzelii*
8	++	9	21	11	*B. afzelii*
9	−/+++	9	10	17	*B. garinii*
10	+++	15	19	12	*B. afzelii*
11	+++	10	14	14	*B. afzelii–B. garinii*
12	+++	8	16	13	*B. afzelii*
13	+++	11	16	11	*B. afzelii*
14	+++	13	17	14	*B. afzelii*
15	+++	7	18	10	*B. afzelii*
16	+++	8	13	13	*B. afzelii–B. garinii*
17	+++	11	18	14	*B. afzelii*
18	++	14	20	14	*B. afzelii*
19	+++	11	15	12	*B. afzelii*
20	+++	14	17	15	*B. afzelii*
21	+++	9	15	10	*B. afzelii*
22	+++	13	15	11	*B. afzelii*
23	−/+++	10	15	12	*B. afzelii*
24	+++	13	18	13	*B. afzelii*
25	+++	10	14	12	*B. afzelii*
Total		271	403	319	

Immunoblot: B, *B. burgdorferi* sensu stricto; A, *B. afzelii*; G, *B. garinii*.

causative agent of ACA. Dual infection with *B. afzelii* and *B. garinii* or reinfection with one or the other genospecies may be suspected in patients reacting with both *B. afzelii* and *B. garinii*. The immunoblot described here also allows the discrimination of false-positive reactions in screening tests and occasionally the confirmation of a chronic Lyme borreliosis in spite of a negative ELISA. It therefore seems to be a sensitive and specific confirmatory test in the laboratory diagnosis of chronic Lyme borreliosis.

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References

1. Steere AC. Lyme disease. N Engl J Med 1989; 321: 586–96.
2. Canica MM, Nato F, du Merle L, Mazie JC, Baranton G, Postic D. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand J Infect Dis 1993; 25: 441–8.
3. Baranton G, Postic D, Saint-Girons I, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov. and group VS 461 associated with Lyme borreliosis. Int J Syst Bacteriol 1992; 42: 378–83.
4. Dressler F, Ackermann R, Steere AC. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. J Infect Dis 1994; 169: 313–18.
5. Anthonissen FM, De Kesel M, Hoet PP, Bigaignon GH. Evidence for the involvement of different genospecies of *Borrelia* in the clinical outcome of Lyme disease in Belgium. Res Microbiol 1994; 145: 327–31.
6. Assous MV, Postic D, Paul G, Névot P, Baranton G. Western blot analysis of sera from Lyme borreliosis patients according to the genomic species of the *Borrelia* strains used as antigens. Eur J Clin Microbiol Infect Dis 1993; 12: 261–8.
7. Péter O, Bretz AG, Postic D, Dayer E. Association of distinct species of *Borrelia burgdorferi* sensu lato with neuroborreliosis in Switzerland. Clin Microbiol Infect 1997; 3: 423–31.
8. Péter O, Bretz AG. Polymorphism of outer surface proteins of *Borrelia burgdorferi* as a tool for classification. Zentralbl Bakteriol 1992; 277: 28–33.
9. Postic D, Assous M, Grimont PAD, Baranton G. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of *rrf*(5S)–*rrl*(23S) intergenic spacer amplicons. Int J Syst Bacteriol 1994; 44: 743–52.
10. Demaerschalk I, Ben Messaoud A, De Kesel M, et al. Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. J Clin Microbiol 1995; 33: 602–8.
11. Rijpkema SGT, Tazelaar DJ, Molkenboer MJCH, et al. Detection of *Borrelia afzelii*, *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* and group VS 116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol infect 1997; 3: 109–16.
12. Schwartz I, Wormser GP, Schwartz JJ, et al. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans skin lesions. J Clin Microbiol 1992; 30: 3082–8.
13. Nocton JJ, Dressler F, Rutledge BJ, et al. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in

synovial fluid from patients with Lyme arthritis. N Engl J Med 1994; 330: 229–34.
14. Wilske B, Fingerle V, Preac-Mursic V, et al. Immunoblot using recombinant antigens derived from different geno-species of *Borrelia burgdorferi* sensu lato. Med Microbiol Immunol 1994; 183: 43–59.
15. Balmelli T, Piffaretti JC. Association between different clinical manifestations of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Res Microbiol 1995; 146: 329–40.

High intraplatelet cGMP levels in human sepsis

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Nitric oxide (NO) is synthesized from L-arginine by a group of enzymes, the nitric oxide synthases (NOS). Under normal circumstances the endothelial constitutive isoform releases small quantities of NO, which acts as an endogenous vasodilator and inhibits platelet adhesion and aggregation [1]. These actions are mediated by the activation of soluble guanylate cyclase which increases the concentration of cyclic guanosine monophosphate (cGMP) in target cells. Expression of the inducible NOS isoform is activated in various cells (endothelium, smooth muscle, macrophages, etc.) in response to inflammatory cytokines and bacterial lipopolysaccharide (LPS). This enzyme synthesizes large quantities of NO which play a significant role in the characteristic hemodynamic changes of septic shock [1]. There are indirect data supporting the idea of increased production of NO during sepsis. High plasma and urine levels of NO_2/NO_3 (inactive metabolic end products of NO) have been demonstrated in sepsis in both animals [2] and humans [3]. Both in endotoxin treated rats [4] and in human septic shock [5], an increase in plasma cGMP levels has been observed.

Keaney et al [6] have recently reported that LPS-induced shock in rabbits increases intraplatelet cGMP levels, reflecting the effect of high amounts of NO on these cells. Intraplatelet cGMP levels are considered to be a good index of the global NO pathway activity [1,7,8]. We hypothesized that the high production of NO in human sepsis should produce an increase in intraplatelet cGMP. We therefore measured intraplatelet cGMP levels in a group of septic patients and correlated these with other clinical and biochemical parameters.

Twelve patients with sepsis (five men and seven women), aged 61 ± 20 years (mean $\pm$ standard deviation), were studied. Patients were eligible for study if they fulfilled the sepsis criteria of the American College of Chest Physician Society and Critical Care Medicine Association [9]. Table 1 shows clinical and biochemical parameters. Patients with acquired immunodeficiency syndrome, active tuberculosis, neoplasia, autoimmune and hematologic diseases, and patients who had received antiplatelet drugs, glucocorticoids, immunosuppressants or antibiotics before the diagnosis, were excluded. The control group comprised 12 age- and sex-matched healthy volunteers (four men and eight women) aged 52 ± 11 years. Informed consent was obtained from all patients and volunteers and the research was approved by a local human investigations committee in accordance with the Helsinki Declaration.

Blood samples for cGMP determinations were collected into 0.105 M sodium citrate from the cubital vein, at diagnosis before starting antibiotic treatment (day 0), and 1 (day 1) and 7 days later (day 7). Plasma and intraplatelet cGMP was measured as described previously [7]. Serum creatinine levels were determined by the Jaffé kinetic method in Autoanalyzer Hitachi 737 (Boehringer Mannheim, Mannheim, Germany).

All data were analyzed using Statistical Package for Social Sciences software. Since the data distribution was non parametric, results are expressed as median and range. The Mann–Whitney rank-sum test was used for the comparisons between groups. Correlations were done with the Spearman test. Differences were considered significant if $p < 0.05$.

As shown in Figure 1, day 0 intraplatelet cGMP levels were higher in the septic patients (0.87 (0.35–5.06) pmol/10^9 platelets (median and range)), than in the control group, 0.23 (0.1–0.4) pmol/10^9 platelets; $p = 0.0001$). Plasma cGMP levels were also significantly increased in the septic patients (4.39 (1.59–13.78) nmol/L), when compared with the control group (1.87 (1.09–4.06) nmol/L, $p = 0.02$). We did not find significant correlations between plasma or intraplatelet cGMP levels and number of platelets, diastolic blood pressure (DBP), systolic blood pressure (SBP), APACHE II score or serum creatinine levels at day 0.

In septic patients intraplatelet cGMP levels were 1.06 (0.19–4.80) pmol/10^9 platelets at day 1, and 0.58 (0.32–2.60) pmol/10^9 platelets at day 7; plasma cGMP levels were 4.94 (2.21–11.96) nmol/L at day 1 and 2.82 (2.39–6.06) nmol/L at day 7. Intraplatelet cGMP levels did not change significantly after antibiotic treatment within the period of time studied. On the contrary, plasma cGMP levels of septic patients at day 7 had reached normal levels.

Our study demonstrates for the first time that intraplatelet cGMP levels are increased in septic